

Chronic Toxicity Testing of the Chevron/Cawelo Water District "Inlet to Reservoir B" C18 Eluate: Assessment of GAC Treatment and Chemical Analyses of the C18 Eluate

Sample collected January 11, 2010

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PACIFIC ECORISK
ENVIRONMENTAL CONSULTING & TESTING

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1. INTRODUCTION

Chevron USA Inc. has contracted Pacific EcoRisk (PER) to perform an evaluation of the chronic toxicity of Chevron/Cawelo Water District (Chevron/Cawelo) "Inlet to Reservoir B" effluent. Previous testing of this effluent has indicated the presence of toxicity to the test species used. Follow-up Toxicity Identification & Evaluations (TIEs) have further indicated that naphthenic acids were a likely cause of the toxicity in these effluents.

Previous chronic toxicity testing of an "Inlet to Reservoir B effluent" sample collected January 11, 2010, indicated significant reductions in fathead minnow survival and growth:

Effects of "Inlet to Reservoir B" Effluent on Fathead Minnows – Initial Testing

There were significant reductions in survival at the $\geq 50\%$ 'Inlet to Reservoir B' effluent concentrations and further significant reductions in growth at the 25% effluent concentration.

In an attempt to better identify the likely cause(s) of this observed toxicity, a "targeted" Phase I Toxicity Identification Evaluation (TIE) was performed. Based upon previous observation of significant toxicity to fathead minnows being removed by the C18 treatment of the 9/21/09 effluent sample, the Phase I TIE of the 1/11/10 effluent sample was "targeted" towards the C18 treatment, and included Graduated pH (pH6, pH7, and pH8) treatments to evaluate possible pH lability of any organic toxicants. Also, as the fathead minnow growth-response in the previous TIE did not provide any additional interpretive value, the TIE of the 1/11/10 effluent sample was limited to evaluation of the survival response.

Results of the Phase I TIE of the "Inlet to Reservoir B" Effluent

As in the initial test of the 1/11/10 effluent sample, there were significant reductions in survival in the untreated (Baseline) effluent, confirming that this toxicity was persistent and present at the time of the TIE.

Key TIE Observations:

- There was pH-labile toxicity, with toxicity increasing as pH decreased to pH6, and toxicity decreasing as pH increased to pH8. This is suggestive of a weakly acidic toxicant that becomes less polar as the pH decreases and more polar as the pH increases. This type of pattern would be consistent with naphthenic acids as a cause of toxicity;
- There was significant removal of survival toxicity by the filtration treatment, which suggests that some fraction of the toxicants present were associated with particulates;
- There was complete removal of any residual toxicity (i.e., toxicity remaining after the filtration treatment) by the C18 treatment, indicating that non-polar organics were a cause of the observed toxicity. **Note – these C18 columns were then frozen for potential further study.**

As previously stated, TIE testing of the 9/21/09 effluent sample had similarly indicated toxicity removal by C18 treatment. Those C18 columns were subsequently sequentially eluted and toxicity was recovered in the 80%, 85%, and 90% methanol eluate fractions. These methanol eluate fractions (and their corresponding blanks) were shipped to Dr. Cliff Lange at Auburn University for chemical analysis targeted to chemicals that are typical constituents of petroleum refinery operations (e.g., volatile organic compounds, naphthenic acids, naphthalenes, phenolics, alkanes, and amines). Most important was the observation of measured concentrations of naphthenic acids in the toxic C18 eluate fractions at concentrations that might be expected to cause toxicity to these aquatic organisms. It is important to note that naphthenic acids also matched up with the TIE profiles, in particular the pH lability in conjunction with toxicity removal by C18.

TIE testing previously performed by PER for other refinery-related effluents has similarly indicated naphthenic acids as commonly-observed causes of refinery effluent toxicity, and follow-up Toxicity Reduction Evaluation (TRE) testing indicated that treatment of the toxic effluents with granulated activated carbon (GAC) was successful in removing the observed toxicity. With that in mind, testing of (untreated) effluent side-by-side with GAC-treated effluent was performed on an "Inlet to Reservoir B" effluent sample collected 9/3/10 to assess the efficacy of GAC treatment in the removal of toxicity from the effluent. The results of that initial assessment of GAC efficacy in toxicity removal from the Inlet to Reservoir B effluent follow:

Efficacy of GAC Treatment in Removing Toxicity from the Inlet to Reservoir B Effluent

There were significant reductions in survival at the $\geq 25\%$ effluent concentrations; and further significant reductions in growth at the 12.5% effluent concentration; after GAC treatment, there were no significant reductions in survival or growth. **Conclusion:** The GAC treatment effectively removed the toxicity from the effluent.

CURRENT TESTING

On that basis, the frozen C18 columns from the earlier TIE of the 1/11/10 effluent sample were thawed out and eluted with methanol, with the eluate being tested for toxicity to fathead minnows as well as efficacy of GAC treatment in removing any toxicity that might be observed. The current chronic toxicity evaluation consisted of performing the US EPA 7-day survival & growth test with larval fathead minnows (*Pimephales promelas*). This report describes the performance and results of this testing.

2. TOXICITY TEST PROCEDURES

The methods used in conducting this chronic toxicity testing followed EPA testing manual "Short-Term Methods for Estimating the Chronic Effects of Effluents and Receiving Waters to Freshwater Organisms, Fourth Edition" (EPA-821-R-02-013).

2.1 C18 Solid Phase Extraction (SPE) Treatment and Column Elution

The C18 TIE treatment test is used to identify effluent toxicity that is due to compounds that are removed or sorbed onto chromatographic resin (i.e., C18 columns) specific for non-polar organic compounds. At the time of the initial TIE of the 1/11/10 effluent sample, 1.0-L aliquots of effluent were passed over each of multiple 1 gm C18 columns. The C18-treated effluent went on to be tested, and the C18 columns were frozen for potential follow-up Phase II TIE work.

For the current testing, frozen C18 columns were removed from the freezer and thawed out to room temperature. The C18 columns were then eluted and the eluate was tested for recovery of toxicity.

The C18 columns were eluted with 100% methanol and the eluate was collected and diluted back up to the 1X effluent concentration for toxicity testing. Method blank columns were similarly eluted. Fathead minnows were tested at the 100% (= 1X) effluent concentration. Additional aliquots of the diluted 1X eluate were set aside and were shipped on ice to Dr. Cliff Lange at Auburn University for chemical analysis.

2.2 Preparation of GAC-Treated Eluate

To prepare the GAC-treated effluents, separatory funnels were loaded with rinsed GAC. Using a peristaltic pump, effluent was pumped up into the bottom of the funnel and passed through the entire column of GAC at a rate of 12 mL/L before flowing out the top (the overall loading rate was 1-L of GAC per treatment of 6-L of 1X eluate). A 'GAC-Treatment Blank' was prepared in a similar fashion using C18 eluate blank medium. The GAC-treated eluate (and method blank) was tested identically to the untreated eluate, as described below.

2.3 Survival and Growth Toxicity Testing with Larval Fathead Minnows

The chronic fathead minnow test consists of exposing larval fish to effluent for 7 days, after which effects on survival and growth are evaluated. The specific procedures used in this testing are described below.

The Lab Water Control for this test consisted of USEPA synthetic moderately-hard water (prepared by addition of ACS-reagent grade chemicals to Type 1 lab water [reverse-osmosis, de-ionized water]). The effluent C18 eluate (and eluate blank) was tested at the 100% concentration only; the GAC-treated effluent eluate (and accompanying blank) was similarly tested at the 100% concentration only. Fresh test solutions were prepared daily. "New" water quality characteristics (pH, D.O., and conductivity) were measured on these test solutions prior to use in the tests.

There were 2 replicates at each test treatment, each replicate consisting of 200 mL of test media in a 400-mL glass beaker. These tests were initiated by randomly allocating 10 larval fathead minnows (<48 hrs old) into each replicate. The replicate beakers were placed in a temperature-controlled room at 25°C, under cool-white fluorescent lighting on a 16L:8D photoperiod. The test fish were fed brine shrimp nauplii twice daily.

Each replicate was examined daily, with any dead animals, uneaten food, wastes, and other detritus being removed. The number of live fish in each replicate was determined and then approximately 80% of the test media in each beaker was carefully poured out and replaced with fresh test solution. "Old" water quality characteristics (pH, D.O., and conductivity) were measured on the old test water that had been discarded from one randomly-selected replicate at each treatment.

After 7 days exposure, the number of live fish in each replicate beaker was recorded. The fish from each replicate were then carefully euthanized in methanol, rinsed in de-ionized water, and transferred to a pre-dried and pre-tared weighing pan. These fish were then dried at 100°C for >24 hrs and re-weighed to determine the total weight of fish in each replicate; the total weight was then divided by the initial number of fish per replicate (n=10) to determine the "biomass value". The resulting survival and growth ("biomass value") data were analyzed to evaluate any impairment(s) caused by the effluents and GAC-treated effluents; all statistical analyses were performed using the CETIS® statistical software.

2.3.1 Reference Toxicant Testing of the Larval Fathead Minnows

In order to assess the sensitivity of the fish to toxic stress, a concurrent reference toxicant test was performed. The reference toxicant test was performed similarly to the effluent test, except that test solutions consisted of Lab Water Control media spiked with NaCl at test concentrations of 0.75, 1.5, 3, 6, and 9 gm/L. The resulting test response data were analyzed to determine key dose-response point estimates (e.g., EC50); all statistical analyses were made using the CETIS® software. These response endpoints were then compared to the "typical response" ranges established by the mean \pm 2 SD of the point estimates generated by the most recent previous reference toxicant tests performed by this lab.

3. TOXICITY TESTING RESULTS

3.1 Effects of 'Inlet to Reservoir B' Eluate on Fathead Minnows

The results of this test are summarized below in Table 2. There was 100% survival and a mean 'biomass value' of 0.34 mg at the Lab Water Control treatment. There were significant reductions in survival in the untreated 100% eluate, indicating that the toxicity that had been observed in the initial testing and previous Phase I TIE of this effluent was still present at the time of the current testing.

After GAC treatment, there were no significant reductions in survival or growth in the C18 eluate. **Conclusion:** The GAC treatment effectively removed the toxicity from the effluent C18 eluate.

The test data and the summary of statistical analyses for this test are presented in Appendix B.

Table 2. Effects of 'Inlet to Reservoir B' effluent C18 eluate on fathead minnows.		
Effluent C18 Eluate Treatment	% Survival	Mean Fish Biomass Value (mg)
Lab Water Control	100	0.34
Eluate Blank	100	0.32
100% Eluate	5*	0.01*
GAC-Treated Eluate Blank	95	0.30
GAC-Treated 100% Eluate	95	0.27

* - The response at this test treatment was significantly less than the Lab Control treatment response.

3.2 Reference Toxicant Toxicity to Fathead Minnows

The results of this test are summarized below in Table 3. There was 80% survival and a mean biomass value of 0.30 mg at the Lab Control treatment. The survival EC₅₀ was 3.7 gm/L NaCl and the growth IC₅₀ was 2.9 gm/L NaCl.

These reference toxicant test results are consistent with the "typical response" ranges established by previous fathead minnow reference toxicant tests performed in this laboratory, indicating that these organisms were responding to toxic stress in a typical fashion.

The test data and summary of statistical analyses for this test are presented in Appendix C.

Table 3. Reference toxicant testing: effects of NaCl on fathead minnows.		
NaCl Treatment (gm/L)	% Survival	Mean Fish Biomass Value (mg)
Lab Control	80	0.30
0.75	82.5	0.31
1.5	90	0.30
3	52.5*	0.14*
6	15*	0.05*
9	0*	0*
Summary of Statistics		
Survival EC ₅₀ or Growth IC ₅₀ =	3.7 gm/L NaCl	2.9 gm/L NaCl

* - The response at this test treatment was significantly less than the Lab Control treatment response at $p < 0.05$.

4. CHEMICAL ANALYSES OF THE TOXIC C18 ELUATE

The effluent eluate and eluate blank were shipped to Dr. Cliff Lange at Auburn University for chemical analysis targeted to chemicals that are typical constituents of petroleum refinery operations (e.g., volatile organic compounds, naphthenic acids, naphthalenes, phenolics, alkanes, and amines). The results of these analyses are summarized in Table 4, below.

Of particular interest are the reported concentrations of **naphthenic acids**. Naphthenic acids are naturally occurring linear and cyclic carboxylic compounds associated with the acidic fraction of petroleum, and are recognized as common causes of aquatic toxicity in petroleum refinery effluents. The cumulative measured concentration of the naphthenic acids included in the analyses of in the C18 eluate was ~5.0 mg/L, which is near the LC50 range reported for aquatic organisms. However, it is important to note that the current analysis was limited to 6 representative compounds, whereas there are over 100 naphthenic acid compounds, suggesting that the concentration of total naphthenic acids in the C18 eluate was much greater than 5.0 mg/L.

Furthermore, it must be noted that in the Phase II TIE C18 elution, methanol is used as the solvent due to the fact the when the eluate is reconstituted to the 1X concentration with Control water, the residual amount of methanol present is below toxicity thresholds. Stronger solvents such as methylene chloride, hexane, etc., would almost certainly have resulted in greater desorption of naphthenic acids from the C18 columns than did methanol, which would have resulted in even higher reported concentrations.

Table 4. Results of targeted chemical analyses of the toxicity C18 column eluates (units = mg/L).		
NAPHTHALENES	Effluent C18 Eluate	Eluate Blank
1-methyl-naphthalene	0.11	0.00
2-methyl-naphthalene	0.13	0.00
1,5-dimethyl-naphthalene	0.11	0.00
1,7-dimethyl-naphthalene	0.03	0.00
naphthalene	0.21	0.01
Total Naphthalenes =	0.59	0.01
NAPHTHENIC ACIDS		
cyclohexanecarboxylic acid	0.97	0.04
methyl,pentyl cyclohexanecarboxylic acid	0.61	0.06
methyl,pentyl cyclopentanecarboxylic acid	0.80	0.03
heptylcyclohexanecarboxylic acid	0.75	0.09
cyclopentanecarboxylic acid	1.13	0.05
diethylcyclopentanecarboxylic acid	0.76	0.04
Total Naphthenic Acids =	5.02	0.31
VOAs		
1,2,4-trimethylbenzene	0.13	0.01
1-ethyl-2-methyl benzene	0.11	0.03
1,3-diethyl benzene	0.16	0.01
1-methyl-3-propyl benzene	0.18	0.02
1-methyl-3-(1-methylethyl)-benzene	0.09	0.01

VOAs (continued)	Effluent C18 Eluate	Eluate Blank
1,2-diethyl benzene	0.14	0.04
benzene	0.23	0.01
toluene	0.59	0.03
p-xylene	0.41	0.02
ethylbenzene	0.32	0.03
1-methyl-2-propyl benzene	0.67	0.01
1,3,5-trimethyl benzene	0.17	0.00
1,2-dimethylbenzene	0.69	0.03
1,3-dimethylbenzene	0.55	0.03
Total VOAs =	4.44	0.28
PHENOLICS		
phenol	1.92	0.12
2-methyl phenol	1.34	0.07
3-methyl phenol	1.08	0.06
3,4-dimethyl phenol	0.73	0.06
3-ethyl phenol	0.33	0.02
aniline	0.41	0.05
Total Phenolics =	5.81	0.38
AMINES		
diethanolamine	0.15	0.02
methylamine	0.34	0.02
ethyl amine	0.26	0.03
ethanol amine	0.11	0.01
triazene	0.00	0.00
methyl diethanol amine	0.19	0.02
ethylenediamine	0.28	0.06
Total Amines =	1.33	0.16
ALKANES		
3-methyl-1-pentene	0.56	0.07
decane	0.70	0.04
2,7-dimethyl octane	0.52	0.05
4-methyl-nonane	0.56	0.09
2,6-dimethyloctane	0.13	0.01
3-ethyl-2methyl-heptane	0.21	0.00
undecane	0.37	0.00
dodecane	0.34	0.02
tridecane	0.48	0.03
tetradecane	0.23	0.02
pentadecane	0.13	0.04
hexadecane	0.22	0.03
heptadecane	0.08	0.01
octadecane	0.05	0.01
nonadecane	0.06	0.03
eicosane	0.08	0.00
heneicosane	0.03	0.00
docosane	0.02	0.01
octacosane	0.02	0.00
dotriacontane	0.04	0.00
tetracontane	0.06	0.01
Total Alkanes =	4.89	0.47

5. SUMMARY AND CONCLUSIONS

Effects of "Inlet to Reservoir B" Effluent on Fathead Minnows

There were significant reductions in survival and growth in the untreated effluent C18 eluate. After GAC treatment, there were no significant reductions in survival or growth in the C18 eluate. **Conclusion:** The GAC treatment effectively removed the toxicity from the effluent C18 eluate.

Chemical Analyses of the Effluent Eluate

The effluent eluate and eluate blank were shipped to Dr. Cliff Lange at Auburn University for chemical analysis targeted to chemicals that are typical constituents of petroleum refinery operations (e.g., VOCs, naphthenic acids, naphthalenes, phenolics, alkanes, and amines). The Lab Report for these analyses is provided as Appendix D.

Of particular interest are the reported concentrations of **naphthenic acids**. Naphthenic acids are naturally occurring linear and cyclic carboxylic compounds associated with the acidic fraction of petroleum, and are recognized as common causes of aquatic toxicity in refinery effluents. The cumulative measured concentration of the naphthenic acids included in the analyses of in the C18 eluate was ~5.0 mg/L, which is near the LC50 range reported for aquatic organisms. However, it is important to note that the current analysis was limited to 6 representative compounds, whereas there are over 100 naphthenic acid compounds; this suggests that the concentration of total naphthenic acids in the C18 eluate was much greater than 5.0 mg/L.

Furthermore, it must be noted that in the Phase II TIE C18 elution, methanol is used as the solvent due to the fact the when the eluate is reconstituted to the 1X concentration with Control water, the residual amount of methanol present is below toxicity thresholds. Stronger solvents such as methylene chloride, hexane, etc., would almost certainly have resulted in greater desorption of naphthenic acids from the C18 columns than did methanol, which would have resulted in even higher reported concentrations.

It must be noted that the discussion of naphthenic acids above should not be construed as a conclusion that naphthenic acids are the proximate cause of the observed toxicity or that any of the other contaminants are not the cause(s) of toxicity. Our contracted scope-of-work was the performance of toxicity testing and facilitation of the chemical analyses. Our discussion of naphthenic acids was provided as anecdotal observations based upon previous work performed by our lab for other refinery clients. If a more complete assessment of the roles of these contaminants as causes of toxicity is needed, then a focused assessment on a contaminant-by-contaminant basis may be warranted.

5.1 QA/QC Summary

Test Conditions – Test conditions (pH, D.O., temperature, etc.) were all within acceptable limits. All analyses were performed according to laboratory Standard Operating Procedures.

Negative Lab Control – The biological responses in the Lab Water Control treatments for these tests were within acceptable limits.

Positive Control – The results of the concurrent reference toxicant test were consistent with the “typical response” ranges established by previous reference toxicant tests performed in our lab, indicating that the test organisms used in the current tests were responding to toxic stress in a typical and consistent fashion.

Appendix A

Chain-of-Custody Record for the Collection and Delivery of the Chevron/Cawelo "Inlet to Reservoir B" Effluent Sample

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CHAIN OF CUSTODY RECORD

PACIFIC ECORISK
 2250 Cordelia Rd
 Fairfield, CA 94534
 Ph: (707) 207-7760
 Fax: (707) 207-7916
 www.pacificecorisk.com

RESULTS TO:

Pacific Ecorisk

BILL TO:

Precision Agriculture
321 19th St.
Bakersfield, CA 93301
 Attn: Account Payable
 Phone: 661-323-1682
 Email: Sharrise@palab-inc.com

PROJECT:

ANALYSES REQUESTED

REMARKS

SAMPLE IDENTIFICATION	DATE	TIME	SAMPLE MATRIX	GRAB/COMP.	# CONTAINERS/TYPE
* INLET TO RES. B	01/11/09	8:30	W	GRAB	20 / PLASTIC
* VALLEY WASTE	1	8:40	1	1	4 / PLASTIC
* OUTLET TO CANAL	1	9:20	1	1	4 / PLASTIC
* PRE-POSO CREEK	1	9:55	1	1	4 / PLASTIC
* SPLITTER BOX	1	11:00	1	1	2 / PLASTIC
* WETLANDS	01/11/09	12:30	1	1	2 / PLASTIC
					1
					1

#1
 #2
 #3
 #4
 #5
 #6

METHOD OF SHIPMENT:

FedEx:

UPS:

HAND:

OTHER:

CONTACT CARRIER

COMMENTS:

CODES:

RELINQUISHED BY: (SIGNATURE)

Sharrise

RECEIVED BY: (SIGNATURE)

Sharrise

PAGE

1355

1/11/10

1901

OF

WHITE - RETURN W/ SAMPLE

YELLOW - KEEP FOR YOUR RECORDS

Appendix B

Test Data for the Evaluation of the Chronic Toxicity of "Inlet to Reservoir B" Eluate to Fathead Minnows

11/2

CETIS Summary Report

Untreated C18 Eluate

Report Date:

14 Dec-10 15:18 (p 1 of 1)

Test Code:

40634 | 13-8177-1171

Chronic Larval Fish Survival and Growth Test

Pacific EcoRisk

Batch ID: 01-8195-8377	Test Type: Growth-Survival (7d)	Analyst: Padrick Anderson
Start Date: 06 Nov-10 15:15	Protocol: EPA-821-R-02-013 (2002)	Diluent: Laboratory Water
Ending Date: 13 Nov-10 09:30	Species: Pimephales promelas	Brine: Not Applicable
Duration: 6d 18h	Source: Chesapeake Cultures, Inc.	Age: 1

Sample ID: 12-5926-3608	Code: Eff	Client: Precision Analytical
Sample Date: 11 Jan-10 07:30	Material: Effluent	Project: 17723
Receive Date: 11 Jan-10 19:01	Source: Precision Analytical	
Sample Age: 299d 8h (1.1 °C)	Station: Inlet Resv B	

Batch Note: Eluate

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
10-0110-7501	7d Survival Rate	<100	100	N/A	14.9%	>1	Equal Variance t Two-Sample Test
12-8774-8648		0	>0		N/A		Fisher Exact Test
02-8254-6458	Mean Dry Biomass-mg	<100	100	N/A	7.8%	>1	Equal Variance t Two-Sample Test
07-3627-2687		0	>0		16.0%		Equal Variance t Two-Sample Test
10-2830-1198	Mean Dry Weight-mg	<100	100	N/A	1.62%	>1	Equal Variance t Two-Sample Test
21-0120-1114		0	>0		16.0%		Equal Variance t Two-Sample Test

7d Survival Rate Summary

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Elution Blank	2	1	1	1	1	1	0	0	0.0%	0.0%
0	Lab Water Contr	2	1	1	1	1	1	0	0	0.0%	0.0%
100		2	0.05	0.0236	0.0764	0	0.1	0.05	0.0707	141.0%	95.0%

Mean Dry Biomass-mg Summary

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Elution Blank	2	0.319	0.309	0.328	0.3	0.337	0.0185	0.0262	8.21%	0.0%
0	Lab Water Contr	2	0.337	0.337	0.338	0.337	0.338	0.0005	0.000708	0.21%	-5.97%
100		2	0.009	0.00425	0.0138	0	0.018	0.009	0.0127	141.0%	97.2%

Mean Dry Weight-mg Summary

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Elution Blank	2	0.319	0.309	0.328	0.3	0.337	0.0185	0.0262	8.21%	0.0%
0	Lab Water Contr	2	0.337	0.337	0.338	0.337	0.338	0.0005	0.000708	0.21%	-5.97%
100		1	0.18			0.18	0.18	0	0	0.0%	43.5%

7d Survival Rate Detail

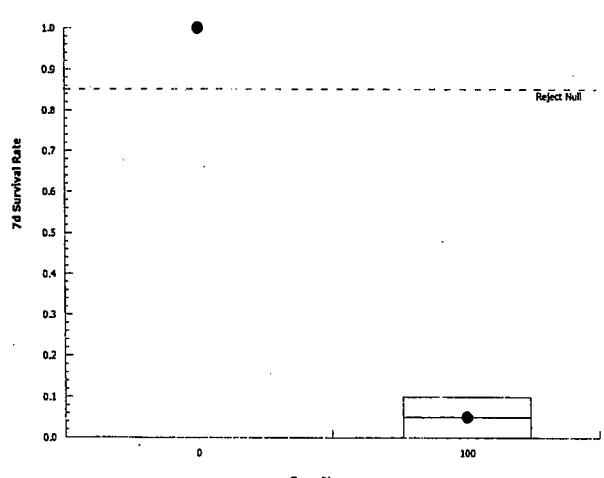
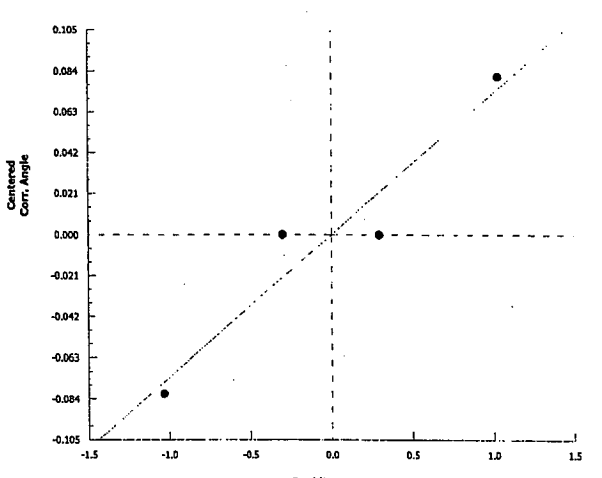
Conc-%	Control Type	Rep 1	Rep 2
0	Elution Blank	1	1
0	Lab Water Contr	1	1
100		0	0.1

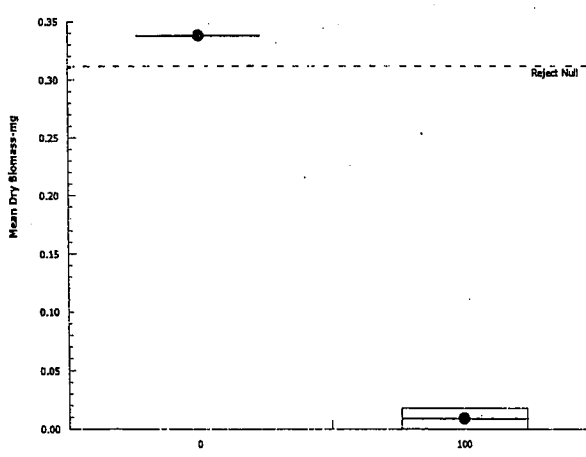
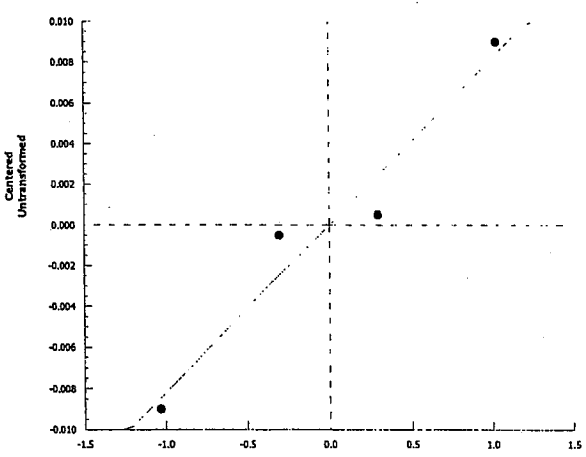
Mean Dry Biomass-mg Detail

Conc-%	Control Type	Rep 1	Rep 2
0	Elution Blank	0.3	0.337
0	Lab Water Contr	0.338	0.337
100		0	0.018

Mean Dry Weight-mg Detail

Conc-%	Control Type	Rep 1	Rep 2
0	Elution Blank	0.3	0.337
0	Lab Water Contr	0.338	0.337
100		0.18	

Chronic Larval Fish Survival and Growth Test							Pacific EcoRisk				
Analysis ID: 10-0110-7501		Endpoint: 7d Survival Rate		CETIS Version: CETISv1.8.0							
Analyzed: 14 Dec-10 15:13		Analysis: Parametric-Two Sample		Official Results: Yes							
Data Transform		Zeta	Alt Hyp	MC Trials	Test Result			PMSD			
Angular (Corrected)		0	C > T	Not Run	Sample fails 7d survival rate endpoint			14.9%			
Equal Variance t Two-Sample Test											
Control	vs	Conc-%	Test Stat	Critical	DF	MSD	P-Value	Decision(α :5%)			
Lab Water Control		100*	14.4	2.92	2	0.238	0.0024	Significant Effect			
ANOVA Table											
Source	Sum Squares		Mean Square		DF	F Stat	P-Value	Decision(α :5%)			
Between	1.373		1.373		1	207	0.0048	Significant Effect			
Error	0.01327967		0.006639833		2						
Total	1.386279		1.37964		3						
Distributional Tests											
Attribute	Test		Test Stat	Critical	P-Value	Decision(α :1%)					
Variances	Mod Levene Equality of Variance		7.66E+15	98.5	<0.0001	Unequal Variances					
Distribution	Shapiro-Wilk W Normality		0.945	-0.335	0.6830	Normal Distribution					
7d Survival Rate Summary											
Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Water Contr	2	1	1	1	1	1	0	0	0.0%	0.0%
100		2	0.05	0.0231	0.0769	0	0.1	0.05	0.0707	141.0%	95.0%
Angular (Corrected) Transformed Summary											
Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Water Cont	2	1.41	1.41	1.41	1.41	1.41	0	0	0.0%	0.0%
100		2	0.24	0.196	0.284	0.159	0.322	0.0815	0.115	48.0%	83.0%
Graphics											
											

Chronic Larval Fish Survival and Growth Test							Pacific EcoRisk				
Analysis ID: 02-8254-6458		Endpoint: Mean Dry Biomass-mg			CETIS Version: CETISv1.8.0						
Analyzed: 14 Dec-10 15:16		Analysis: Parametric-Two Sample			Official Results: Yes						
Data Transform		Zeta	Alt Hyp	MC Trials	Test Result			PMSD			
Untransformed		0	C > T	Not Run	Sample fails mean dry biomass-mg endpoint			7.8%			
Equal Variance t Two-Sample Test											
Control	vs	Conc-%	Test Stat	Critical	DF	MSD	P-Value	Decision(α:5%)			
Lab Water Control		100*	36.4	2.92	2	0.0263	0.0004	Significant Effect			
ANOVA Table											
Source		Sum Squares		Mean Square		DF	F Stat	P-Value	Decision(α:5%)		
Between		0.107912		0.107912		1	1330	0.0008	Significant Effect		
Error		0.0001625152		8.125762E-05		2					
Total		0.1080745		0.1079932		3					
Distributional Tests											
Attribute		Test		Test Stat	Critical	P-Value	Decision(α:1%)				
Variances		Variance Ratio F		323	16200	0.0707	Equal Variances				
Distribution		Shapiro-Wilk W Normality		0.967	-0.335	0.8245	Normal Distribution				
Mean Dry Biomass-mg Summary											
Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Water Contr	2	0.337	0.337	0.338	0.337	0.338	0.000498	0.000704	0.21%	0.0%
100		2	0.009	0.00416	0.0138	0	0.018	0.009	0.0127	141.0%	97.3%
Graphics											
											

CETIS Summary Report

GAC-Treated C18 Eluate

Report Date:

14 Dec-10 15:39 (p 1 of 1)

Test Code:

40634 | 19-5168-7085

Chronic Larval Fish Survival and Growth Test

Pacific EcoRisk

Batch ID: 00-3694-2618	Test Type: Growth-Survival (7d)	Analyst: Padrick Anderson
Start Date: 06 Nov-10 15:15	Protocol: EPA-821-R-02-013 (2002)	Diluent: Laboratory Water
Ending Date: 13 Nov-10 09:30	Species: Pimephales promelas	Brine: Not Applicable
Duration: 6d 18h	Source: Chesapeake Cultures, Inc.	Age: 1

Sample ID: 11-7289-6631	Code: Eff	Client: Precision Analytical
Sample Date: 11 Jan-10 07:30	Material: Effluent	Project: 17723
Receive Date: 11 Jan-10 19:01	Source: Precision Analytical	
Sample Age: 299d 8h (1.1 °C)	Station: Inlet Resv B	

Batch Note: Eluate + GAC

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
08-8768-3474	7d Survival Rate	100	>100	N/A	14.9%	1	Equal Variance t Two-Sample Test
14-7811-9408		0	>0		14.9%		Equal Variance t Two-Sample Test
07-9472-0699	Mean Dry Biomass-mg	100	>100	N/A	21.3%	1	Equal Variance t Two-Sample Test
17-0381-3657		0	>0		22.1%		Equal Variance t Two-Sample Test
06-7322-1276	Mean Dry Weight-mg	0	>0		17.0%		Equal Variance t Two-Sample Test
12-6592-7914		100	>100	N/A	17.0%	1	Equal Variance t Two-Sample Test

7d Survival Rate Summary

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	GAC Blank	2	0.95	0.924	0.976	0.9	1	0.05	0.0707	7.44%	0.0%
0	Lab Water Contr	2	1	1	1	1	1	0	0	0.0%	-5.26%
100		2	0.95	0.924	0.976	0.9	1	0.05	0.0707	7.44%	0.0%

Mean Dry Biomass-mg Summary

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	GAC Blank	2	0.301	0.293	0.31	0.286	0.317	0.0155	0.0219	7.27%	0.0%
0	Lab Water Contr	2	0.319	0.309	0.328	0.3	0.337	0.0185	0.0262	8.21%	-5.64%
100		2	0.274	0.267	0.281	0.26	0.288	0.0141	0.0199	7.25%	9.1%

Mean Dry Weight-mg Summary

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	GAC Blank	2	0.317	0.317	0.318	0.317	0.318	0.000389	0.00055	0.17%	0.0%
0	Lab Water Contr	2	0.319	0.309	0.328	0.3	0.337	0.0185	0.0262	8.21%	-0.35%
100		2	0.288	0.288	0.289	0.288	0.289	0.000394	0.000557	0.19%	9.1%

7d Survival Rate Detail

Conc-%	Control Type	Rep 1	Rep 2
0	GAC Blank	0.9	1
0	Lab Water Contr	1	1
100		1	0.9

Mean Dry Biomass-mg Detail

Conc-%	Control Type	Rep 1	Rep 2
0	GAC Blank	0.286	0.317
0	Lab Water Contr	0.3	0.337
100		0.288	0.26

Mean Dry Weight-mg Detail

Conc-%	Control Type	Rep 1	Rep 2
0	GAC Blank	0.318	0.317
0	Lab Water Contr	0.3	0.337
100		0.288	0.289

Chronic Larval Fish Survival and Growth Test

Pacific EcoRisk

Analysis ID: 08-8768-3474

Endpoint: 7d Survival Rate

CETIS Version: CETISv1.8.0

Analyzed: 14 Dec-10 15:38

Analysis: Parametric-Two Sample

Official Results: Yes

Data Transform	Zeta	Alt Hyp	MC Trials	Test Result	PMSD
Angular (Corrected)	0	C > T	Not Run	Sample passes 7d survival rate endpoint	14.9%

Equal Variance t Two-Sample Test

Control	vs	Conc-%	Test Stat	Critical	DF	MSD	P-Value	Decision(α :5%)
Lab Water Control		100	1	2.92	2	0.238	0.2113	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α :5%)
Between	0.006639833	0.006639833	1	1	0.4226	Non-Significant Effect
Error	0.01327967	0.006639833	2			
Total	0.0199195	0.01327967	3			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α :1%)
Variances	Mod Levene Equality of Variance	65500	98.5	<0.0001	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.945	-0.335	0.6830	Normal Distribution

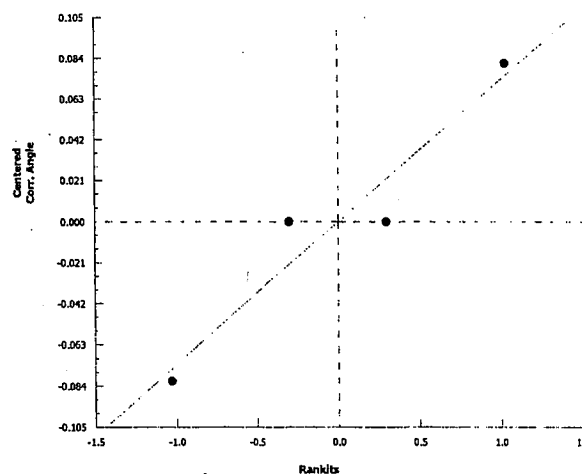
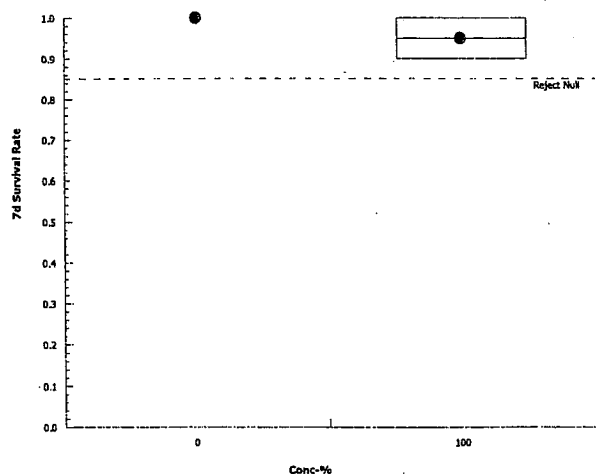
7d Survival Rate Summary

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Water Contr	2	1	1	1	1	1	0	0	0.0%	0.0%
100		2	0.95	0.923	0.977	0.9	1	0.05	0.0707	7.44%	5.0%

Angular (Corrected) Transformed Summary

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Water Cont	2	1.41	1.41	1.41	1.41	1.41	0	0	0.0%	0.0%
100		2	1.33	1.29	1.37	1.25	1.41	0.0815	0.115	8.66%	5.77%

Graphics



Chronic Larval Fish Survival and Growth Test

Pacific EcoRisk

Analysis ID: 07-9472-0699

Endpoint: Mean Dry Biomass-mg

CETIS Version: CETISv1.8.0

Analyzed: 14 Dec-10 15:38

Analysis: Parametric-Two Sample

Official Results: Yes

Data Transform	Zeta	Alt Hyp	MC Trials	Test Result	PMSD
Untransformed	0	C > T	Not Run	Sample passes mean dry biomass-mg endpoint	1.3%

Equal Variance t Two-Sample Test

Control	vs	Conc-%	Test Stat	Critical	DF	MSD	P-Value	Decision(α :5%)
Lab Water Control		100	1.91	2.92	2	0.0678	0.0979	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α :5%)
Between	0.001975768	0.001975768	1	3.66	0.1958	Non-Significant Effect
Error	0.001079359	0.0005396794	2			
Total	0.003055127	0.002515447	3			

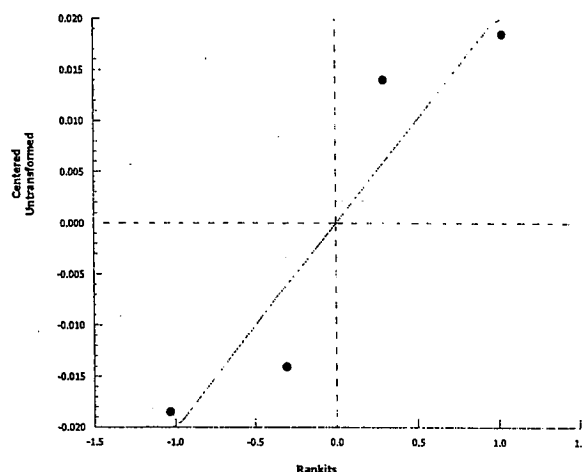
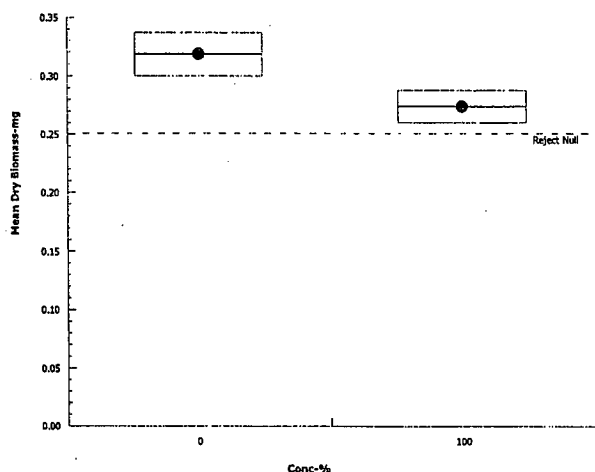
Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α :1%)
Variances	Variance Ratio F	1.73	16200	0.8270	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.84	-0.335	0.1942	Normal Distribution

Mean Dry Biomass-mg Summary

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Water Contr	2	0.319	0.309	0.328	0.3	0.337	0.0185	0.0262	8.21%	0.0%
100		2	0.274	0.266	0.282	0.26	0.288	0.0141	0.0199	7.25%	14.0%

Graphics



7 Day Chronic Fathead Minnow Toxicity Test Data

Client: Precision Analytical
 Test Material: Inlet to Reservoir B
 Test ID#: 40634 Project #: 17723
 Test Date: 11-6-10

Organism Log#: 25191 Age: 24h
 Organism Supplier: ABS
 Control/Diluent: EPAMH
 Control Water Batch: 1336

Treatment	Temp (°C)	pH		D.O. (mg/L)		Conductivity (µs/cm)	# Live Organisms				SIGN-OFF
		New	Old	New	Old		A	B			
Lab Control	25.4	8.19		8.6		318	10	10			Date: 11-6-10
Eluate Blank	25.4	8.16		9.5		315	10	10			Test Solution Prep: FA
100% Eluate	25.4	8.10		9.6		316	10	10			New WQ: SC
Eluate + GAC Blank	25.4	7.97		9.4		259	10	10			Initiation Time: 1515
Eluate + GAC	25.4	7.75		9.4		256	10	10			Initiation Signoff: A
											Sample ID: 23374
Meter ID	30A	PH09		RD03		EC04					
Lab Control	25.2	8.12	8.14	8.8	7.9	320	10	10			Date: 11/7/10
Eluate Blank	25.2	7.97	8.04	10.0	7.8	312	10	10			Test Solution Prep: SH
100% Eluate	25.2	7.99	8.03	10.1	8.0	313	10	10			New WQ: SV
Eluate + GAC Blank	25.2	7.34	7.35	9.5	8.1	243.2	10	10			Renewal Time: 1130
Eluate + GAC	25.2	7.00	7.19	9.6	8.1	252.7	10	10			Renewal Signoff: JPL
											Old WQ: SV
Meter ID	30A	PH03	PH03	RD03	RD03	EC03					
Lab Control	25.0	8.35	7.99	9.4	8.2	314	10	10			Date: 11/8/10
Eluate Blank	25.0	8.21	7.76	11.4	6.8	313	10	10			Test Solution Prep: DW
100% Eluate	25.0	8.08	7.76	11.3	7.0	313	10	9			New WQ: MD
Eluate + GAC Blank	25.0	7.43	7.35	11.0	7.6	255	10	10			Renewal Time: 0930
Eluate + GAC	25.0	7.26	7.10	10.7	6.9	253	10	10			Renewal Signoff: DW
											Old WQ: SV
Meter ID	30A	PH04	PH09	RD04	RD04	EC05					
Lab Control	25.2	8.25	8.01	9.0	5.3	318	10	10			Date: 11.9.10
Eluate Blank	25.2	8.18	7.85	10.9	5.4	306	10	10			Test Solution Prep: EKK
100% Eluate	25.2	8.13	7.82	12.4	5.9	246	9	7			New WQ: EKK
Eluate + GAC Blank	25.2	8.25	7.60	12.4	6.4	252	10	10			Renewal Time: 1400
Eluate + GAC	25.2	7.70	7.37	12.7	5.7	312	10	10			Renewal Signoff: MW
											Old WQ: JY
Meter ID	30A	PH14	PH09	RD04	RD05	EC05					

7 Day Chronic Fathead Minnow Toxicity Test Data

Client: Precision Analytical
 Test Material: Inlet to Reservoir B
 Test ID#: 40634 Project #: 17723
 Test Date: 11-6-10 Randomization: NA

Organism Log#: 25191 Age: <24 hrs
 Organism Supplier: ABS
 Control/Diluent: EPAMH
 Control Water Batch: 1336
 Fraction: 0

Treatment	Temp (°C)	pH		D.O. (mg/L)		Conductivity (µs/cm)	# Live Organisms				SIGN-OFF
		new	old	new	old		A	B			
Lab Control	25.3	8.56	8.03	8.5	2.8	320	10	10			Date: 11-10-10
Eluate Blank	25.3	8.44	7.94	9.9	2.6	312	10	10			Test Solution Prep: AL
100% Eluate	25.3	8.33	7.51	9.9	1.4	315	3	2			New WQ: <u>W</u>
Eluate + GAC Blank	25.3	8.51	7.51	9.9	5.8	248	10	10			Renewal Time: 13:30
Eluate + GAC	25.3	8.41	7.32	9.7	2.0	253	10	10			Renewal Signoff: AL
											Old WQ: <u>W</u>
Meter ID	48A	PH14	PH14	R005	R005	EC03					
Lab Control	25.3	8.23	7.94	9.4	7.6	307	10	10			Date: 11-11-10
Eluate Blank	25.3	7.96	7.84	11.0	6.0	307	10	10			Test Solution Prep: AL
100% Eluate	25.3	7.92	7.67	11.1	5.1	312	1	2			New WQ: <u>SVV</u>
Eluate + GAC Blank	25.3	7.92	7.23	11.3	7.5	246.2	10	10			Renewal Time: 11:10
Eluate + GAC	25.3	7.75	7.22	10.9	7.0	252.3	10	10			Renewal Signoff: <u>MM</u>
											Old WQ: <u>SVV</u>
Meter ID	48A	PH03	PH03	R004	R004	EC05					
Lab Control	24.8	8.57	8.32	9.1	8.5	318	10	10			Date: 11-12-10
Eluate Blank	24.8	8.45	8.07	10.3	6.8	307	10	10			Test Solution Prep: <u>MM</u>
100% Eluate	24.8	8.24	8.02	10.2	7.2	325	0	1			New WQ: <u>CC</u>
Eluate + GAC Blank	24.8	8.14	7.83	10.9	7.3	249.5	9	10			Renewal Time: 11:30
Eluate + GAC	24.8	7.90	7.54	10.7	7.5	249.2	10	9			Renewal Signoff: <u>PA</u>
											Old WQ: <u>CC</u>
Meter ID	48A	PH14	PH03	R005	R003	EC04					
Lab Control	24.9		8.20		5.1	338	10	10			Date: 11-13-10
Eluate Blank	24.9		7.86		5.8	345	10	10			Termination Time: 0930
100% Eluate	24.9		7.81		5.6	344	-	1			Termination Signoff: <u>PA</u>
Eluate + GAC Blank	24.9		7.85		5.8	275	9	10			Old WQ: <u>NVS</u>
Eluate + GAC	24.9		7.37		6.5	289	10	9			
Meter ID	48A		PH03		R005	EC05					

Fathead Minnow Dry Weight Data Sheet

Client: Precision Analytical , Test ID #: 40634 Project # 17723
 Sample: Inlet to Reservoir B Tare Weight Date: 11/6/10 Sign-off: [Signature]
 Test Date: ~~11/13/10~~ 11.6.10 Final Weight Date: 11/17/10 Sign-off: [Signature]

Pan ID	Treatment	Replicate	Initial Pan Weight (mg)	Final Pan Weight (mg)	Initial # of Organisms	Biomass Value (mg)
1	Lab Control	A	181.84	184.84	10	0.300
2		B	168.62	171.99	10	0.337
3	Eluate Blank	A	186.37	189.75	10	0.338
4		B	163.81	167.218	10	0.337
5	100% Eluate	A	166.57	—	—	—
6		B	172.65	172.83	10	0.018
7	Eluate + GAC	A	173.99	176.85	10	0.286
8	Blank	B	174.67	177.84	10	0.28 0.327
9	Eluate + GAC	A	181.29	184.10	10	0.288
10		B	176.98	179.58	10	0.260
QA1			185.40	185.40		
Balance ID:			#1	#1		

Appendix C

Test Data and Summary of Statistics for the Reference Toxicant Evaluation of the Fathead Minnows

CETIS Summary Report

 Report Date: 24 Nov-10 10:19 (p 1 of 2)
 Test Code: 40635 | 02-9408-1320

Chronic Larval Fish Survival and Growth Test							Pacific EcoRisk
Batch ID:	11-0047-6335	Test Type:	Growth-Survival (7d)			Analyst:	Padrick Anderson
Start Date:	06 Nov-10 15:30	Protocol:	EPA-821-R-02-013 (2002)			Diluent:	Laboratory Water
Ending Date:	13 Nov-10 10:00	Species:	Pimephales promelas			Brine:	Not Applicable
Duration:	6d 18h	Source:	Chesapeake Cultures, Inc.			Age:	1
Sample ID:	04-1854-4806	Code:	NaCl			Client:	Pacific Ecorisk
Sample Date:	06 Nov-10 15:30	Material:	Sodium chloride			Project:	17724
Receive Date:	06 Nov-10 15:30	Source:	Reference Toxicant				
Sample Age:	N/A (25.4 °C)	Station:	In House				
Comparison Summary							
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
09-2783-6448	7d Survival Rate	1.5	3	2.121	27.9%		Dunnett's Multiple Comparison Test
19-9022-1316	Mean Dry Biomass-mg	1.5	3	2.121	27.9%		Dunnett's Multiple Comparison Test
11-9125-8327	Mean Dry Weight-mg	6	>6	N/A	35.5%		Dunnett's Multiple Comparison Test
Point Estimate Summary							
Analysis ID	Endpoint	Level	g/L	95% LCL	95% UCL	TU	Method
08-2994-1963	7d Survival Rate	EC10	2.13	1.74	2.46		Linear Regression (MLE)
		EC15	2.36	1.97	2.69		
		EC20	2.57	2.18	2.9		
		EC25	2.76	2.37	3.09		
		EC40	3.3	2.93	3.63		
		EC50	3.68	3.32	4.01		
08-4855-7672	Mean Dry Biomass-mg	IC5	1.58	N/A	1.78		Linear Interpolation (ICPIN)
		IC10	1.72	N/A	2.03		
		IC15	1.87	N/A	2.36		
		IC20	2.01	0.849	2.66		
		IC25	2.16	1.31	2.97		
		IC40	2.6	1.83	4.02		
16-9043-4162	Mean Dry Weight-mg	IC5	1.06	N/A	2.97		Linear Interpolation (ICPIN)
		IC10	1.37	N/A	N/A		
		IC15	1.82	N/A	N/A		
		IC20	2.37	N/A	N/A		
		IC25	2.93	0.596	N/A		
		IC40	>6	N/A	N/A		
		IC50	>6	N/A	N/A		

CETIS Summary Report

Report Date:

24 Nov-10 10:19 (p 2 of 2)

Test Code:

40635 | 02-9408-1320

Chronic Larval Fish Survival and Growth Test											Pacific EcoRisk
7d Survival Rate Summary											
Conc-g/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Water Contr	4	0.8	0.77	0.83	0.7	0.9	0.0408	0.0816	10.2%	0.0%
0.75		4	0.825	0.761	0.889	0.6	1	0.0854	0.171	20.7%	-3.12%
1.5		4	0.9	0.847	0.953	0.7	1	0.0707	0.141	15.7%	-12.5%
3		4	0.525	0.469	0.581	0.4	0.7	0.075	0.15	28.6%	34.4%
6		4	0.15	0.128	0.172	0.1	0.2	0.0289	0.0577	38.5%	81.3%
9		4	0	0	0	0	0	0	0		100.0%
Mean Dry Biomass-mg Summary											
Conc-g/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Water Contr	4	0.303	0.274	0.333	0.212	0.4	0.0394	0.0788	26.0%	0.0%
0.75		4	0.306	0.295	0.317	0.267	0.333	0.0149	0.0297	9.7%	-0.99%
1.5		4	0.298	0.286	0.309	0.251	0.316	0.0155	0.0311	10.4%	1.9%
3		4	0.141	0.112	0.169	0.063	0.223	0.0385	0.0771	54.9%	53.7%
6		4	0.0472	0.0364	0.0581	0.021	0.089	0.0146	0.0292	61.7%	84.4%
9		4	0	0	0	0	0	0	0		100.0%
Mean Dry Weight-mg Summary											
Conc-g/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Water Contr	4	0.378	0.349	0.407	0.265	0.444	0.039	0.0779	20.6%	0.0%
0.75		4	0.381	0.357	0.404	0.3	0.445	0.032	0.064	16.8%	-0.78%
1.5		4	0.333	0.325	0.341	0.312	0.359	0.0113	0.0227	6.81%	11.8%
3		4	0.252	0.223	0.281	0.158	0.319	0.0389	0.0778	30.8%	33.2%
6		4	0.311	0.265	0.358	0.2	0.445	0.0624	0.125	40.1%	17.6%
7d Survival Rate Detail											
Conc-g/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4						
0	Lab Water Contr	0.8	0.8	0.9	0.7						
0.75		0.9	0.8	1	0.6						
1.5		1	1	0.9	0.7						
3		0.6	0.7	0.4	0.4						
6		0.1	0.2	0.1	0.2						
9		0	0	0	0						
Mean Dry Biomass-mg Detail											
Conc-g/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4						
0	Lab Water Contr	0.212	0.322	0.4	0.279						
0.75		0.325	0.333	0.3	0.267						
1.5		0.312	0.316	0.311	0.251						
3		0.188	0.223	0.088	0.063						
6		0.021	0.089	0.039	0.04						
9		0	0	0	0						
Mean Dry Weight-mg Detail											
Conc-g/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4						
0	Lab Water Contr	0.265	0.403	0.444	0.399						
0.75		0.361	0.416	0.3	0.445						
1.5		0.312	0.316	0.346	0.359						
3		0.313	0.319	0.22	0.158						
6		0.21	0.445	0.39	0.2						

7 Day Chronic Fathead Minnow Reference Toxicant Test Data

Client: Reference Toxicant 40049 n Log#: 25191 Age: 424h
 Test Material: Sodium Chloride Organism Supplier: ABS
 Test ID#: 40635 Project #: 17724 Control/Diluent: EPAMH
 Test Date: 11-8-10 Randomization: 4.6.1 Control Water Batch: 1336

Treatment (g/L)	Temp (°C)	pH		D.O. (mg/L)		Conductivity (µs/cm)	# Live Organisms				SIGN-OFF
		New	Old	New	Old		A	B	C	D	
Control	25.4	8.02		9.0		327	10	10	10	10	Date: <u>11-8-10</u>
0.75	25.4	7.98		9.0		1944	10	10	10	10	Test Solution Prep: <u>TWL</u>
1.5	25.4	7.93		9.0		3320	10	10	10	10	New WQ: <u>SG</u>
3	25.4	7.80		9.1		6120	10	10	10	10	Initiation Time: <u>1530</u>
6	25.4	7.84		9.1		11150	10	10	10	10	Initiation Signoff: <u>2/TWL</u>
9	25.4	7.80		9.1		14970	10	10	10	10	RT Stock Batch #: <u>66</u>
Meter ID	30A	PH14		RD14		EC03					
Control	25.4	8.09	8.19	8.6	7.5	325	9	9	9	9	Date: <u>11/7/10</u>
0.75	25.4	8.02	8.07	8.7	7.5	1784	10	10	10	8	Test Solution Prep: <u>DW</u>
1.5	25.4	7.97	8.02	8.9	7.3	3240	10	10	10	9	New WQ: <u>SMH</u>
3	25.4	7.94	7.92	9.0	7.4	5940	10	10	10	10	Renewal Time: <u>4015 1115</u>
6	25.4	7.89	7.82	9.1	7.3	11190	4	10	7	10	Renewal Signoff: <u>SMH</u>
9	25.4	-	7.78	-	7.4	-	0	0	0	0	Old WQ: <u>SW</u>
Meter ID	30A	PH14	PH03	RD04	RD03	EC03					RT Stock Batch #: <u>66</u>
Control	25.0	8.25	8.01	9.3	6.9	316	9	8	9	8	Date: <u>11/8/10</u>
0.75	25.0	8.12	7.88	9.5	6.6	1736	9	8	10	6	Test Solution Prep: <u>DW</u>
1.5	25.0	8.03	7.86	9.4	7.2	3140	10	10	9	9	New WQ: <u>MO</u>
3	25.0	7.98	7.83	9.8	7.4	6020	9	10	9	9	Renewal Time: <u>1030</u>
6	25.0	7.84	7.79	10.3	7.3	11190	2	10	5	8	Renewal Signoff: <u>SMH</u>
9	-	-	-	-	-	-	-	-	-	-	Old WQ: <u>SW</u>
Meter ID	30A	PH09	PH09	RD04	RD04	EC05					RT Stock Batch #: <u>66</u>
Control	25.4	8.17	7.36	9.1	5.1	318	9	8	9	7	Date: <u>11.9.10</u>
0.75	25.4	8.11	7.40	8.9	5.0	1581	9	8	10	6	Test Solution Prep: <u>EWL</u>
1.5	25.4	8.03	7.47	9.1	4.7	3160	10	10	9	9	New WQ: <u>SMH</u>
3	25.4	7.97	7.46	9.4	4.8	6010	9	10	9	9	Renewal Time: <u>12:55</u>
6	25.4	7.91	7.48	9.7	4.3	11330	2	8	4	6	Renewal Signoff: <u>TWL</u>
9	-	-	-	-	-	-	-	-	-	-	Old WQ: <u>SW</u>
Meter ID	30A	PH14	PH09	RD04	RD05	EC05					RT Stock Batch #: <u>66</u>

7 Day Chronic Fathead Minnow Reference Toxicant Test Data

Client: Reference Toxicant
 Test Material: Sodium Chloride
 Test ID#: 40635 Project #: 17724
 Test Date: 11/9/10 Randomization: 4.6.1

Organism Log#: 25191 Age: 224hrs
 Organism Supplier: ABS
 Control/Diluent: EPAMH
 Control Water Batch: 1336

Treatment (g/L)	Temp (°C)	pH		D.O. (mg/L)		Conductivity (µs/cm)	# Live Organisms				SIGN-OFF
		new	old	new	old		A	B	C	D	
Control	25.3	8.04	8.06	8.5	6.8	322	9	8	9	7	Date: 11/10/10
0.75	25.3	8.01	7.88	8.7	6.3	1898	9	8	10	6	Test Solution Prep: <u>SW</u>
1.5	25.3	7.90	7.81	8.8	6.7	3390	10	10	9	9	New WQ: <u>SW</u>
3	25.3	7.95	7.74	9.0	6.7	6120	9	9	8	9	Renewal Time: 10:00
6	25.3	7.90	7.68	9.2	8.3	11180	1	4	3	4	Renewal Signoff: <u>SW</u>
9	25.3	7.86	-	9.7	-	14900	-	-	-	-	Old WQ: <u>SW</u>
Meter ID	30A	pH14	pH14	R005	R005	EC03					RT Stock Batch #: 66
Control	25.3	8.31	8.13	8.6	7.5	323	8	8	9	7	Date: 11-11-10
0.75	25.3	8.23	7.97	8.7	7.6	1799	9	8	10	6	Test Solution Prep: <u>TWL</u>
1.5	25.3	8.18	7.89	8.9	7.8	3420	10	10	9	7	New WQ: <u>SW</u>
3	25.3	8.13	7.80	9.0	7.7	6060	9	8	5	7	Renewal Time: 11:20
6	25.3	8.07	7.71	9.5	7.2	11400	1	3	2	2	Renewal Signoff: <u>TWL</u>
9	-	-	-	-	-	-	-	-	-	-	Old WQ: <u>SW</u>
Meter ID	48A	pH12	pH03	R005	R004	EC03					RT Stock Batch #: 66
Control	25.0	8.29	8.00	8.9	7.8	317	8	8	9	7	Date: 11-12-10
0.75	25.0	8.17	7.88	8.8	7.8	2052	9	8	10	6	Test Solution Prep: <u>TWL</u>
1.5	25.0	8.13	7.80	9.0	7.6	3120	10	10	9	7	New WQ: <u>SW</u>
3	25.0	8.06	7.75	9.2	7.7	5880	9	7	5	5	Renewal Time: 11:55
6	25.0	7.97	7.70	9.5	7.5	11210	1	3	1	2	Renewal Signoff: <u>TWL</u>
9	-	-	-	-	-	-	-	-	-	-	Old WQ: <u>SW</u>
Meter ID	48A	pH03	pH14	R003	R004	EC03					RT Stock Batch #: 66/67
Control	25.1		8.17		6.6	326	8	8	9	7	Date: 11-13-10
0.75	25.1		7.87		6.8	2066	9	8	10	6	Termination Time: 10:00
1.5	25.1		7.73		6.5	3370	10	10	9	7	Termination Signoff: <u>SW</u>
3	25.1		7.68		6.7	6120	6	7	4	4	Old WQ: <u>SW</u>
6	25.1		7.64		7.7	11590	1	2	1	2	
9	-		-		-	-	-	-	-	-	
Meter ID	48A		pH09		R004	EC04					

Fathead Minnow Dry Weight Data Sheet

Client: Reference Toxicant Test ID #: 40635 Project #: 17724
 Sample: Sodium Chloride Tare Weight Date: 11/6/10 Sign-off: CS
 Test Date: 11.4.10 Final Weight Date: 11/17/10 Sign-off: CS

Pan ID	Concentration	Replicate	Initial Pan Weight (mg)	Final Pan Weight (mg)	Initial # of Organisms	Biomass Value (mg)
1	Control	A	165.19	167.31	10	0.212
2		B	179.83	183.05	10	0.322
3		C	189.74	193.74	10	0.400
4		D	179.23	182.02	10	0.279
5	0.75	A	171.86 76	175.01	10	0.325
6		B	169.96	173.29	10	0.333
7		C	185.21	188.21	10	0.300
8		D	163.50	166.17	10	0.267
9	1.5	A	175.84	178.96	10	0.312
10		B	165.95	169.11	10	0.316
11		C	167.17	170.28	10	0.311
12		D	177.83	180.34	10	0.251
13	3	A	174.53	176.41	10	0.188
14		B	164.54	166.77	10	0.232 0.223
15		C	173.52	174.40	10	0.088
16		D	168.28	168.91	10	0.063
17	6	A	187.09	187.30	10	0.021
18		B	170.65	171.54	10	0.089
19		C	181.61	182.00	10	0.039
20		D	179.35	179.75	10	0.040
21	9	A	177.95	—	10	—
22		B	170.07	—	10	—
23		C	174.31	—	10	—
24		D	166.13	—	10	—
QA1			166.11	166.53		
QA2			169.13	169.13		
QA3			184.14	184.14		
Balance ID:			#1	#1		

Appendix D

Laboratory Report for Chemical Analyses of Effluent C18 Elutriate

211>

IL

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November 29, 2010

Scott Ogle, Ph.D.
Pacific EcoRisk, Inc.
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Fairfield, CA 94534
PH: 707-207-7762 (direct)
PH: 707-207-7760 (general

Re: Refinery Hydrocarbons in two Eluent Samples

Two (2) samples of eluent from were forwarded from Pacific Ecorisk to Dr. Cliff Lange. Samples were extracted using methylene chloride and concentrated to twenty times the initial concentration using a rotary evaporator. Naphthenic acids we analyzed using BF₃/Methanol derivatization followed by GC-FID analysis. Phenols, alkanes, and aromatics were analyzed using EPA Method 625. The amounts of phenolic compounds, alkanes, naphthenic acids, and surfactants, were determined by gas chromatography and are summarized in Table 1.

QA/QC data is presented in Table 2. In general, all check samples were within(plus/minus) ten percent of the actual value.

Warmest Regards,

Dr. Cliff Lange

Table 1. Results of analysis of two Eluent Samples

Pacific Ecorisk	CC Refinery Eluate Eff 1X	CC Refinery Eluate BLK 1X
Sample Date 11-5-10		
Units	mg/L	mg/L
Aromatics		
1,2,4-trimethylbenzene	0.13	0.01
1-ethyl-2-methyl benzene	0.11	0.03
1,3-diethyl benzene	0.16	0.01
1-methyl-3-propyl-benzene	0.18	0.02
1-methyl-3-(1-methylethyl)-benzene	0.09	0.01
1,2-diethyl benzene	0.14	0.04
benzene	0.23	0.01
toluene	0.59	0.03
p-xylene	0.41	0.02
ethylbenzene	0.32	0.03
1-methyl-2-propyl benzene	0.67	0.01
Phenol	1.92	0.12
2-methyl phenol	1.34	0.07
3-methyl phenol	1.08	0.06
1,2-dimethylbenzene	0.69	0.03
1,3-dimethylbenzene	0.55	0.03
3,4-dimethyl phenol	0.73	0.06
3-ethyl phenol	0.33	0.02
Analine	0.41	0.05
1,3,5-trimethyl benzene	0.17	0.00
1-methyl-naphthalene	0.11	0.00
2-methyl-naphthalene	0.13	0.00
1,5-dimethyl-naphthalene	0.11	0.00
1,7-dimethyl-naphthalene	0.03	0.00
Naphthalene	0.21	0.01
NAPHTHENIC ACIDS		
Cyclohexanecarboxylic acid	0.97	0.04
Methyl-pentyl cyclohexanecarboxylic acid	0.61	0.06
Methyl,pentylcyclopentanecarboxylic acid	0.80	0.03
Heptylcyclohexanecarboxylic acid	0.75	0.09
Cyclopentanecarboxylic acid	1.13	0.05
Diethylcyclopentanecarboxylic acid	0.76	0.04
Alkanes		
3-methy-1-pentene	0.56	0.07
Decane	0.70	0.04

2,7-dimethyl octane	0.52	0.05
4-methyl-nonane	0.56	0.09
2,6-dimethyloctane	0.13	0.01
3-ethyl-2methyl-heptane	0.21	0.00
Undecane	0.37	0.00
Dodecane	0.34	0.02
Tridecane	0.48	0.03
Tetradecane	0.23	0.02
Pentadecane	0.13	0.04
Hexadecane	0.22	0.03
Heptadecane	0.08	0.01
Octadecane	0.05	0.01
Nonadecane	0.06	0.03
Eicosane	0.08	0.00
Heneicosane	0.03	0.00
Docosane	0.02	0.01
Octacosane	0.02	0.00
Dotriacontane	0.04	0.00
Tetracontane	0.06	0.01
AMINES		
Diethanolamine	0.15	0.02
Methylamine	0.34	0.02
ethyl amine	0.26	0.03
Ethanol amine	0.11	0.01
Triazene	0.00	0.00
Methyl diethanol amine	0.19	0.02
Ethylenediamine	0.28	0.06

Table 2. QA/QC: Results of Check Samples

QA/QC DATA					
Pacific EcoRisk Samples					
Units	mg/L	mg/L	mg/L	Check Sample	
	Methanol Blank	1.0 mg/L check sample	0.1 mg/L Check sample		
1,2,4-trimethylbenzene	< 0.01	1.06	-	1	
1-ethyl-2-methyl benzene	< 0.01	1.09	-	1	
1,3-diethyl benzene	< 0.01	1.07	-	1	
1-methyl-3-propyl benzene	< 0.01	1.03	-	1	
1-methyl-3-(1-methylethyl)-benzene	< 0.01	0.99	-	1	
1,2-diethyl benzene	< 0.01	1.03	-	1	
benzene	< 0.01	1.08	-	1	
toluene	< 0.01	1.09	-	1	
p-xylene	< 0.01	1.11	-	1	
ethylbenzene	< 0.01	1.05	-	1	
1-methyl-2-propyl benzene	< 0.01	1.09	-	1	
Phenol	0.012	1.13	-	1	
2-methyl phenol	< 0.01	1.05	-	1	
3-methyl phenol	< 0.01	1.04	-	1	
1,2-dimethylbenzene	< 0.001	-	0.099	2	
1,3-dimethylbenzene	< 0.001	-	0.109	2	
3,4-dimethyl phenol	< 0.01	1.07	-	1	
3-ethyl phenol	< 0.01	1.12	-	1	
Aniline	< 0.01	1.08	-	1	
1,3,5-trimethyl benzene	< 0.01	-	0.108	2	
1-methyl-naphthalene	< 0.001	-	0.099	2	
2-methyl-naphthalene	< 0.001	-	0.105	2	
1,5-dimethyl-naphthalene	< 0.001	-	0.107	2	
1,7-dimethyl-naphthalene	< 0.001	-	0.101	2	
Naphthalene	< 0.001	-	0.095	2	
NAPHTHENIC ACIDS					
Cyclohexanecarboxylic acid	< 0.005	1.09	0.107	3,4	
Methyl-pentyl cyclohexanecarboxylic acid	< 0.005	0.98	0.101	3,4	
Methyl,pentylcyclopentanecarboxylic acid	< 0.005	1.06	0.104	3,4	
Heptylcyclohexanecarboxylic acid	< 0.005	0.94	0.096	3,4	
Cyclopentanecarboxylic acid	< 0.005	1.04	0.110	3,4	
Diethylcyclopentanecarboxylic acid	0.006	1.07	0.108	3,4	

3-methyl-1-pentene	< 0.01	1.00	-		
Decane	< 0.01	0.96	-		
2,7-dimethyl octane	< 0.01	0.96	-	5	
4-methyl-nonane	< 0.01	1.04	-	5	
2,6-dimethyloctane	< 0.01	0.97	-	5	
3-ethyl-2methyl-heptane	< 0.01	0.93	-	5	
undecane	< 0.01	0.98	-	5	
dodecane	< 0.01	1.03	-	5	
tridecane	< 0.01	0.95	-	5	
tetradecane	< 0.01	0.93	-	5	
Pentadecane	< 0.01	1.02	-	5	
hexadecane	< 0.01	0.93	-	5	
heptadecane	< 0.01	0.97	-	5	
octadecane	< 0.01	1.03	-	5	
nonadecane	< 0.01	1.05	-	5	
eicosane	< 0.01	1.02	-	5	
heneicosane	< 0.01	1.04	-	5	
docosane	< 0.01	0.97	-	5	
octacosane	< 0.01	1.01	-	5	
dotriacontane	< 0.01	1.02	-	5	
tetracontane	< 0.01	0.95	-	5	
diethanolamine	< 0.01	1.09	-	6	
methylamine	< 0.01	1.11	-	6	
ethyl amine	< 0.01	1.08	-	6	
Ethanol amine	< 0.01	1.10	-	6	
Triazene	< 0.01	1.03	-	6	
Methyl diethanol amine	< 0.01	1.07	-	6	
Ethylenediamine	< 0.01	1.09	-	6	

Check Samples

- 1 ppm mixture of refinery aromatics in 99% methanol.
- 0.1 ppm mixture of refinery aromatics in 99% methanol
- 1.0 ppm Naphthenic acid mixture in 99% methanol
- 0.1 ppm Naphthenic acid mixture in 99% methanol
- 1.0 ppm Alkanes in 99% methanol
- 1.0 ppm Refinery Amines in 99% methanol